A Nuclear Polyhedrosis Virus of the Lawn Armyworm, Spodoptera mauritia (Boisduval) (Lepidoptera: Noctuidae)[1]

YOSHINORI TANADA

LABORATORY OF INSECT PATHOLOGY
DEPARTMENT OF BIOLOGICAL CONTROL
UNIVERSITY OF CALIFORNIA, BERKELEY

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Slightly over two years after the lawn armyworm, *Spodoptera mauritia* (Boisduval), was first recorded in Hawaii, a nuclear polyhedrosis of this insect was discovered by Bianchi (1957). This virus disease plays an important role in the natural control of the lawn armyworm (Tanada and Beardsley, 1958). According to Tanada and Beardsley (1957), the nuclear polyhedrosis virus may have entered Hawaii together with its host. However, no reference has been found by the author to any polyhedrosis disease of the lawn armyworn outside of Hawaii. A disease of this armyworm in South India was considered by Anantanarayanan and Ramakrishna Ayyar (1937) as probably of bacterial origin, but the symptoms recorded by them resemble somewhat those of the nuclear polyhedrosis described in the present report. The descriptions and characteristics of the virus and the disease in the lawn armyworm are presented below.

The study of the pathogenesis of the nuclear polyhedrosis was conducted with larvae reared individually in sterilized, half-pint cardboard containers that were covered with a half of a sterilized petri dish. These larvae were fed mainly tender young shoots of napier grass, *Pennisetum purpureum* Schumach. The temperature in the laboratory ranged in general between 72° F. (22° C.) and 85° F. (29° C.) with occasional extremes of 68° F. (20° C.) and 86° F. (30° C.). The relative humidity was ordinarily 56 to 85 per cent, with extremes of 50 and 92 per cent.

For the histopathological study, the larvae were fixed in alcoholic Bouin's solution (Duboscq-Brasil's modification) and prepared for sectioning through a methyl benzoate and benzene series and thence into paraffin (see Romeis,

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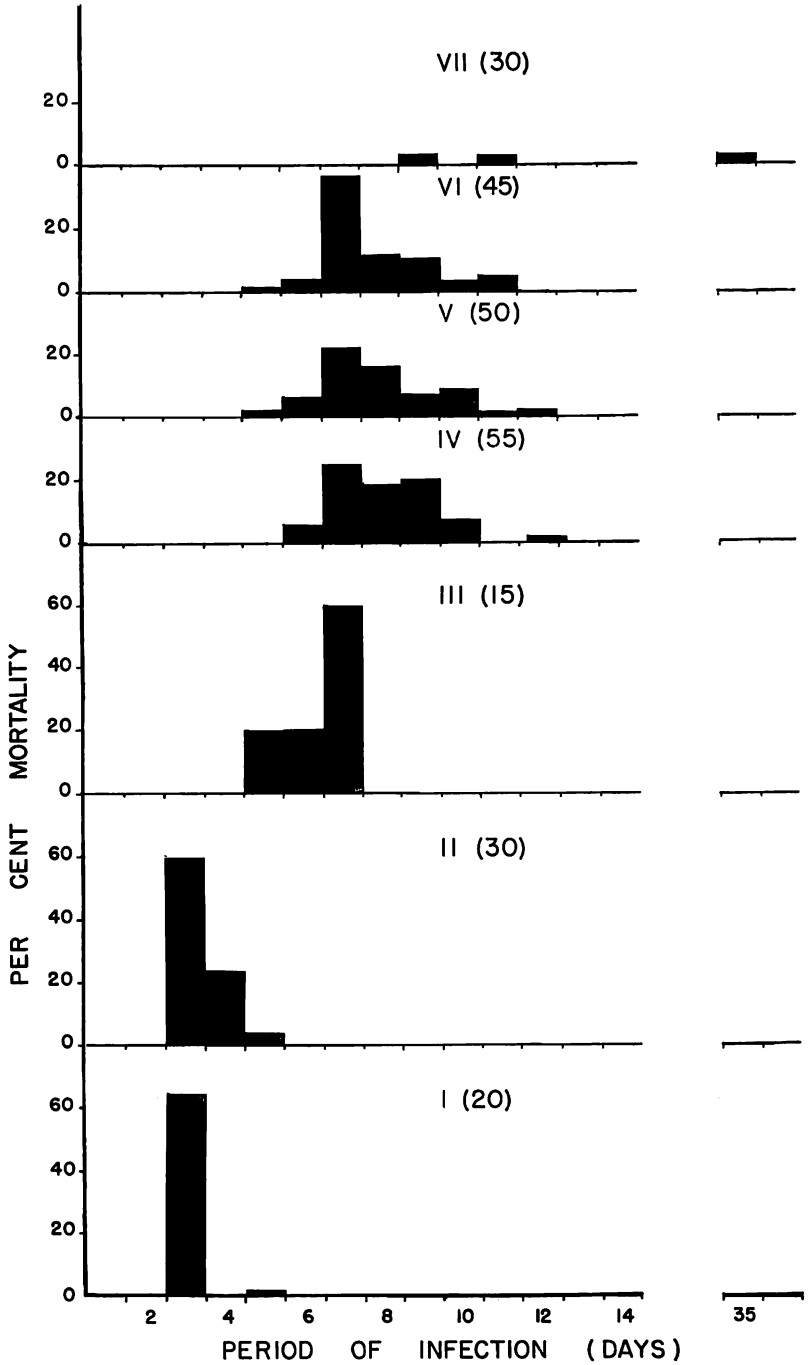
[1] Portions of the study were conducted at the Department of Entomology, University of Hawaii Agricultural Experiment Station, and the study is presented with the approval of the Director as Technical Paper No. 452.

FIG. 1. The period of lethal infection of nuclear polyhedrosis virus in the larvae of *Spodoptera mauritia* as affected by the time of inoculation in the different instars. Roman numerals indicate larval instars, and Arabic numerals in parentheses the total number of larvae tested in each instar.


VII (30)
VI (45)
V (50)
IV (55)
III (15)
II (30)
I (20)
PER CENT MORTALITY
PERIOD OF INFECTION (DAYS)
2
4
6
8
10
12
14
35

1948). The larvae were sectioned longitudinally at 8 μ and stained with Heidenhain's iron-hematoxylin and eosin.

The polyhedra were purified by repeated centrifugations and washings with distilled water. The virus particles were obtained by dissolving the ploy hedra with 0.1 M. Na_2CO_3 and purified by several centrifugations and washings with distilled water. For the electron microscope study, a drop of the polyhedra or virus suspension was dried on the electron microscope grid, shadowed with palladium, and then observed with a RCA electron microscope, type EMU-2B.

The larvae shortly after succumbing to the virus show the typical symptoms of nuclear polyhedrosis, (*i.e.*, their skins become fragile and their internal contents a fluid mass, a typical "wilt" condition). Larvae which become infected in their early instars and die before the fourth instar usually begin to turn pale 2 to 4 days prior to death and, at death, have a whitish or creamy appearance. After death the larvae rapidly darken in color. Older infected larvae which die in the fifth or older instars gradually turn slightly pale with a pinkish tinge several days before death, but otherwise remain nearly as dark brown as the healthy larvae. Thus, in older larvae, change in coloration is not as marked as in the younger larvae.

For the determination of the period of lethal infection (*i.e.*, the period from exposure to the virus until death) massive doses of suspensions of polyhedra on grass leaves were fed to the larvae. The period of lethal infection varied with the larval instars (fig. 1). When infected in the first instar most of the larvae died in 3 days; when in the second instar most of them died in 3 to 4 days; when in the third instar they died in 5 to 7 days; in the fourth to sixth instars most died in 7 to 10 days; when in the seventh instar the larvae were fairly resistant to infection, and the few that were infected died in 9 days or longer. In general, the first 6 larval instars were highly susceptible to the virus, but the last instar seemed to be fairly resistant (only 3 out of 30 larvae were infected in two separate tests).

In infected mature larvae the histopathology is generally similar to that of most nuclear polyhedroses in other insects. The main sites of infection are the hypodermis, fat body and tracheal matrix. Occasionally the nuclei of the striated muscle and of the cells surrounding the nerve cord are infected. At times a few cells in other tissues and organs, such as the ganglia, brain, blood, the secretory portion of the salivary gland, the epithelia of the fore-and hindguts, and the cellular sheath around the testes may contain polyhedra within their nuclei. Nuclei of a few midgut cells located near the junction of the mid- and hindguts were also observed to be infected in one specimen.

The diameter of 50 polyhedra that were measured varied from about 1.07 μ to 3.22 μ with a mean of 1.61 μ, the standard error of which was $\pm$ 0.07 μ (fig. 2A). Widths of 30 naked virus rods ranged from 56.7 mμ to 66.2 mμ, with a mean

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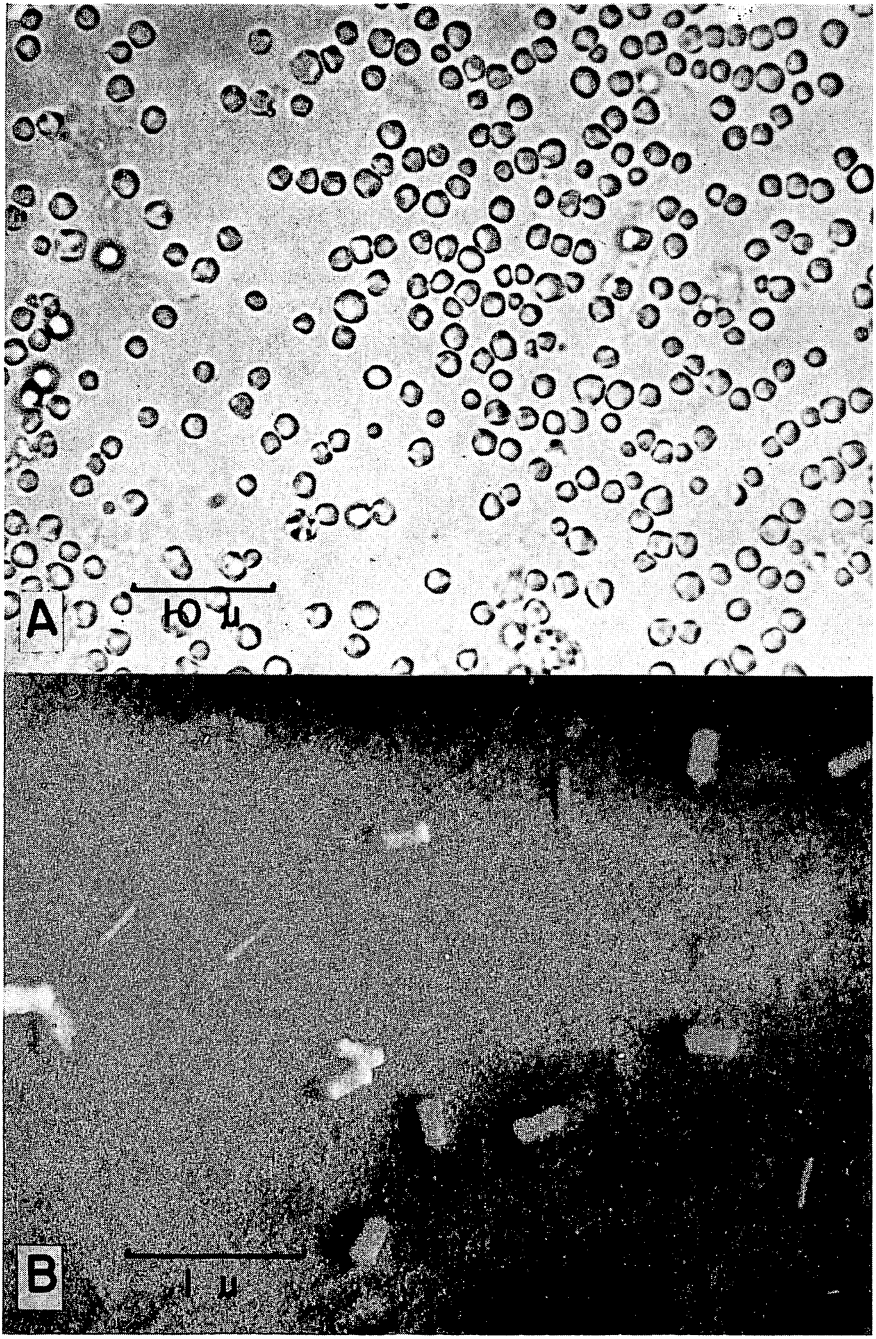
FIG. 2. Nuclear polyhedrosis virus of the lawn armyworm, *Spodoptera mauritia*. A, Polyhedra suspended in water. Photograph taken under an oil immersion planachromat objective (n.a. 1.25) of a Carl Zeiss photomicroscope with built-in automatic timing device. B, Electron micrograph of free virus rods showing individual rods and bundles of rods.


A
10 μ
B
1 μ

of 61.9 mμ and a standard error of ± 0.59 mμ; their lengths varied from 298.1 mμ to 364.3 mμ with a mean of 324.7 ± 3.37 mμ (fig. 2B).

Cross infectivity tests were conducted with the nuclear polyhedrosis virus of *Spodoptera mauritia* and with the nuclear polyhedrosis and granulosis viruses of the cosmopolitan armyworm, *Pseudaletia unipuncta* (Haworth). In the first test the polyhedra of *S. mauritia* were fed to 25 second-instar larvae of *P. unipuncta*, and an equal number of untreated larvae were maintained as controls. None of the larvae died from virus infection. In the second test the polyhedra of *S. mauritia* were fed alone and in combination with the capsules (granulosis virus) of *P. unipuncta* to fourth-instar larvae of *P. unipuncta*. None of the treated or control larvae died with polyhedra within them. On the other hand, 10 control larvae of *P. unipuncta* fed a mixture of polyhedra and capsules both from *P. unipuncta* died of nuclear polyhedrosis. Each treated group contained 15 larvae.

Two tests on the infectivity of the nuclear polyhedrosis and granulosis viruses of *P. unipuncta* were conducted with the lawn armyworm. The viruses were fed alone and combined together. Third-instar larvae were used in both tests. In the first test all groups contained 15 larvae, and in the second all contained 12 larvae, except the control which contained 10. None of the treated or control larvae died from either virus disease.

Inasmuch as the virus is rod-shaped and occurs within a polyhedral inclusion body which develops in the nucleus of the host cell, it belongs to the genus *Borrelinavirus* (see Bergold 1958).

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